(× 492). Fig. 11. Micrasterias decemdentata var. (?) turgidum Taylor (× 492). Fig. 12. Micrasterias decemdentata var. (?) turgidum Taylor, end view (× 368). Fig. 13. Micrasterias arcuata Bailey var. gracilis W. and G. S. West (× 492).

Explanation of Plate 330

Figs. 1, 2. Staurastrum vestitum Ralfs (× 860). Figs. 3, 4. Staurastrum concinnum W. and G. S. West, 4 (× 737); 3 (× 614). Fig. 5. Micrasterias mahabuleshwalensis Hobson (× 270). Figs. 6, 7. Micrasterias triangularis Wolle, 6 (× 270); 7 (× 127). Fig. 8. Staurastrum elongatum Barker (× 450). Figs. 9, 10. Staurastrum striolatum (Naeg.) Archer, zygospore (× 614). Fig. 11. Micrasterias radiosa var. ornata Nordst. (× 270). Figs. 12, 13. Staurastrum furcatum (Ehr.) de Breb. (× 614). Fig. 14. Staurastrum vestitum Ralfs (× 360).

OBSERVATIONS ON THE CYTOLOGY OF SEBACINA GLOBOSPORA N. SP.[1]

R. M. Whelden

Plate 331

In the course of an examination of a great many specimens of Tremellaceous fungi, the writer has found several of particular interest. One collection[2] was noticeable for several reasons, first because the young fruit bodies were found emerging from the ostioles of perithecia of a *Diaporthe* growing in small twigs of *Fraxinus sp.*, suggesting that the *Sebacina* might possibly be parasitic on the *Diaporthe;* second, because mounts in lacto-phenol-cotton blue solution showed extreme irregularity in the orientation of the septa of the basidia, raising the question of whether the nuclear phenomena here would be out of the ordinary; and finally, because it was impossible to identify the fungus as one already described. While the material obtained is a single collection and hence offers little opportunity to determine the range of variation, nevertheless the species appears within limits to be remarkably uniform and seems to justify the conclusion that it represents a new species.

The total collection, comprising some sixty separate fruit bodies, was received in a partially dried condition. A part of the material was at once placed in a moist chamber; after about six hours, part of the fruit bodies were removed and fixed, some in a chrom-acetic solution and some in a mercuric chloride-picric acid solution; eighteen hours later the rest of the material was removed from the moist cham-

[1] Contribution No. 137 from the Laboratories of Cryptogamic Botany of Harvard University.

[2] The fungus, collected at Louisville, Kentucky by Mr. Billy Lee, was sent by him to Dr. D. H. Linder, to whom my thanks are due for the pleasure of studying it.

ber and fixed in the same manner as the first, some thirty fruit bodies in all being thus fixed. This material was embedded, half of it in

Text-fig. SEBACINA GLOBOSPORA, n. sp.

celloidin and half in paraffin, and stained in the manner which the writer has described elsewhere (1).

The young fructifications of this fungus appear as small hemispherical hyaline objects emerging from the ostioles of perithecia of *Diaporthe;* with age they spread out one square centimeter or more in a somewhat irregular fashion over the surface of the bark, to which they are closely pressed. On drying the fruit bodies become distinctly chalky. The mycelium of young fruit bodies seems to originate in the hymenial layer of the perithecium of *Diaporthe* and to grow up through the ostiole, at the mouth of which the hyphae spread fan-wise (TEXT-FIG.). All hyphae comprising this portion of the fruit body, as well as those spreading over the bark in older bodies, are of binucleated segments of great length (FIG. 1). At the tip of each segment branching frequently occurs, especially after the hyphae have emerged from the ostiole. Near the distal, somewhat enlarged part of the segment, the two nuclei, from 2 to 2.5 μ in diameter, almost invariably lie close together, surrounded by a slightly denser region of cytoplasm. Near the surface of the fruit body the hyphal segments, still binucleate, become much shorter, twisted (FIG. 2), and frequently branched, while, in the subhymenial layer the segments are very short and straight (FIG. 3). The cross-walls of these short segmented hyphae frequently show a very evident central thickness which is accentuated by its staining black with haematoxylin. Fusions between two hyphae occur infrequently, but no clamp connections were observed.

At the surface of the fruit body there are cut off binucleate hyphal tips of which the protoplasmic content is much denser. These, the hypobasidial initials, rapidly increase in size, while at the same time the dikaryons unite to form the fusion nucleus, which quickly enlarges to its maximum size of 5 to 6 μ. During this enlargement the chromatin substance becomes more and more definitely aggregated into somewhat irregular elongate masses, which, as the nucleus approaches its maximum size, contract rapidly to form eight distinct small chromosomes (FIGS. 8–10, 13). These chromosomes are short cylindrical objects which seem to show certain distinct differences in size, differences which may be somewhat exaggerated because of the orientation of the chromosomes. At this time the conspicuous nucleolus begins to disappear, as does also the nuclear membrane, leaving the chromosomes centrally located in the protoplasm of the now fully enlarged hypobasidium, with an average measurement of 17.2 x 14.2 μ. Throughout the development of the hypobasidium there has been little change in the appearance of its cytoplasm, which has become somewhat thinner and progressively more vacuolate, especially after nuclear division has occurred. Frequently there are seen scattered through it small granules which stain deeply with haematoxylin (FIGS. 7, 8).

Nuclear division immediately results in the formation of two small daughter nuclei, which may occupy any position in the hypobasidium, although usually they are midway in it (FIGS. 12, 33). One or both of these nuclei may immediately divide again so that one often sees hypobasidia, in which the three or four nuclei are arranged without order (FIGS. 19, 20).

The formation of the first longitudinal septum may occur immediately after the first division of the fusion nucleus (FIGS. 14, 18), or it may be delayed until a much later stage of development; the time of appearance of the second longitudinal septum is equally variable. The direction of these septa of the hypobasidia differs greatly, ranging from the usual longitudinal ones to diagonal ones at every conceivable angle, with frequent cases in which they are oriented at right angles to the long axis of the hypobasidium (FIGS. 21, 25). Not infrequently the first septum divides the hypobasidium transversely in two equal parts, while the second septum cuts one of these sections longitudinally in two, the other remaining without septation (FIG. 26).

The development of the epibasidia likewise shows the greatest irregularity. In the species of *Sebacina* previously studied by the writer, the two to four epibasidia normally developed simultaneously from the apical portion of the hypobasidium. In the present species one often sees hypobasidia showing this characteristic development, the two, rarely more, epibasidia growing directly upward to the surface of the "jelly," expanding slightly or not at all (FIG. 17). However, much more frequently the epibasidia develop very irregularly. The variation from the normal condition may be one of direction only, the two epibasidia growing out at wide angles one to the other, a condition particularly noticeable near the edge of the fruit body (FIG. 5, left). Much more frequent are irregularities in time of appearance of the epibasidia; for example the epibasidium of one of the segments of the hypobasidium may develop only slightly in advance of the other, as in FIG. 22, or may complete its development to spore formation while the other segment shows no indication of such, as in FIG. 15; as a result of such irregularities one frequently observes hypobasidia with one half empty (FIG. 27) and partially (FIG. 23) or completely collapsed (FIG. 34). Even more striking are the hypobasidia in which septum formation is transverse rather than longitudinal; in these either the upper (FIG. 25) or the lower half (FIG. 21) may have discharged its contents in spore formation, while the other half remains unchanged. Frequently the empty portion shows that it was divided independently of the other as in FIG. 26.

That nuclear divisions occur independently of these changes may be seen in such cases as those shown in fig. 25, where the nucleus in the lower portion of the hypobasidium is in the early prophase of division, or more obviously in FIG. 35, where the four chromosomes are definitely present just before the disappearance of the nucleolus and nuclear membrane in metaphase. Nor does any correlation seem to exist between the nuclear condition and the epibasidia, as for example in FIG. 21, where two small epibasidial bulges appear before the single nucleus present in the segment of the hypobasidium shows any sign of division. As the development of the epibasidium progresses, the protoplasm of the hypobasidium becomes more and more vacuolate. The epibasidia themselves vary from the slender form with a uniform diameter from 1 to 1.3 μ of FIG. 33 to a very much swollen type (FIG. 15) in which the maximum diameter is 5 μ. All epibasidia contract rather abruptly at the "jelly" surface to form the slender sterigma at the tip of which the spore is formed.

The spores enlarge rapidly and when mature are subspherical to spherical bodies usually about 8 μ in diameter; rare individuals occur having dimensions of 7.8 x 7 μ to a maximum of 9 x 8 μ: all have a pronounced apiculus 1 μ or slightly more in length (FIG. 28). They often germinate immediately by a single germ tube (FIGS. 29–32), which attains a length of 6–8 μ before forming the secondary spore, similar to, but smaller than, the primary basidiospore. The same variations in form obtain in these germ tubes as occurred in the epibasidia, some being slender and slightly twisting (FIG. 10), others very coarse and straight, (FIG. 32), while still others are considerably inflated and 4 to 4.5 μ in diameter in the middle (FIGS. 31, 32). Germination of the secondary spores was not observed.

From the distal end of the hyphal segment immediately below the hypobasidium one or more branches form soon after the hypobasidium is cut off (FIGS. 7, 12). Two nuclei soon move into the developing branch after which it is cut off by a cross-wall, and becomes another hypobasidium (FIGS. 4, 16). In rare cases the lateral branch appears from the beginning to be somewhat vacuolate; in living bodies these branches are seen to contain many large drops of a refractive substance which, however, in the process of embedding and staining is dissolved out, so that they appear vacuolate (FIGS. 10, 24). These gloeocystidia, as the vacuolate structures may be classified, are always binucleate, although frequently the position of the nuclei, close together one above the other, gives the appearance of a uninucleate condition. What may eventually become of these objects as growth of the fruit body continues was not observed. Other branches start as do these, but grow to a considerable length (FIGS. 11, 23) without noticeable change in diameter or in content: these are probably merely vegetative hyphae characteristic of rapidly growing fruit bodies.

The writer, having already discussed at considerable length the work which has been done on the Tremellaceous fungi (1), does not feel that any long discussion is necessary here. It is to be noted, however, that several workers have reported the occurrence of irregular septation in the basidia of this group, some of them finding therein points indicating the origin of the group; yet so far as the writer knows, there has been no study of the cytological condition involved. With material of this sort available, it seemed of value to ascertain whether any irregularity in nuclear behavior accompanied the more obvious irregularities of septation. The present study indicates that up to the time of septation in the hypobasidium, development is practically identical with that observed in all other members of the group which have been studied. However, both the time and direction of septum formation, as well as the subsequent development of the epibasidia show great irregularity. There at once appear three possible explanations for these irregularities: first is the fact that the fungus before study was revived in a moist chamber; however, the writer has used that method many times in studying other species of *Sebacina,* and only rarely found any irregular forms. Again it may be suggested that the growth of the fruit bodies apparently parasitic on an Ascomycete is significant; while this might account for the variations from normal, there seems to be no real reason why it should. Finally it would seem to the writer more probable that the cause for the irregularities is inherent in the fungus itself. Certainly all the fruit bodies studied in this collection agree in showing the variations, indicating that it is apparently characteristic of the species. Because of this and of the more important fact that the dimensions of its parts, notably the hypobasidia and spores, are considerably larger than those of the species it most closely resembles, *Sebacina* (*Bourdotia*) *cinerella* Bourd. et Galz., the writer feels justified in proposing it as a new species of *Sebacina:*

SEBACINA **globospora,** sp. nov., fruit bodies at first hemispherical and colorless, becoming watery-gray effuse bodies from 6 to 12 mm. in extent, on drying becoming chalky, pressed against but not adnate to the substratum. Hyphae 2 to 3 μ in diameter, without clamp connections. Basidia ovate, 15–17–20 x 12–14–16 μ; average: 17.2 x 14.2 μ.

Epibasidia variable, up to 14 μ in length, 2–3–(5) μ in diameter. Spores spherical to subspherical, 7.8 to 8 μ in diameter, each with a pronounced apiculus. Gloeocystidia rare, 28–35 x 7 μ when mature. Young fruit bodies growing from the ostioles of the perithecia of *Diaporthe*, older ones spreading out over the surface of bark of twigs of *Fraxinus;* collected near Louisville, Kentucky, in September, 1934. (Type in Farlow Herbarium, Harvard University.)

Sebacina **globospora** sp. nov. Fructificationes primo subglobosae decoloresque, tardius cinereae, hyalinae, effusae, 6–12 mm. extensis, exsiccatae cretaceae, depressae sed non substrato adnatae. Hyphae 2–3 μ diam., anodosae. Basidia ovata, 15–17–20 x 12–14–16 μ (plus-minusve 17.2 x 14.2 μ). Epibasidia inconstantia, ad 14 μ longa, 2–3–(5) μ diam. Sporae globosae vel subglobosae, hyalinae, leves, 7.8–8 μ diam., evidenter apiculatae. Gloeocystidia rara, clavata, 28–35 x 7 μ.

Summary

Sebacina globospora, sp. nov., growing from the ostioles of and apparently parasitizing the perithecia of *Diaporthe*, is of particular interest because of the irregularities in the formation of the septa of the hypobasidia, and the time of development of epibasidia. The septa are formed in almost every conceivable position in the hypobasidium. Variation in epibasidial development ranges from the simultaneous formation of the two to four epibasidia to those cases in which one epibasidium has borne a mature spore before the others have appeared at all. The tendency towards suppression of branching as shown in Figs. 5 and 16 immediately calls to mind the condition existing in *Sirobasidium*, in which genus the branching habit has been totally suppressed, to be replaced by a basipetal formation of basidia.

Explanation of Plate 331

All figures drawn with the aid of a Camera lucida at a magnification of about 3700 ×, and reduced in reproduction to about 0.3 ×. An absolute scale is included in the plate.

Figure 1. Elongate mycelial segment in older portions of fruit body. 2. Mycelial segment near surface of fruit body. 3. In sub-hymenial region. 4. Binucleate hypobasidial initial (right) and young hypobasidium (left). 5. Widely diverging epibasidia from a hypobasidium near margin of fruit body, also showing suppression of branching. 6. Hypobasidium with fusion nucleus showing linear masses of chromatin. 7. Basidia developed from single branch tip near center of fruit body. 8. Contraction of chromatin masses. 9 & 10. Eight chromosomes and nucleolus within the nuclear membrane; lateral branch in figure 10 showing early appearance of gloeocystidium. 11 & 12. Hypobasidium containing four and two small nuclei respectively. 13. Hypobasidium containing eight chromosomes and nucleolus in late prophase of fusion nucleus. 14. First longitudinal septum forming after first division of nucleus. 15. A basidium with one epibasidium completely developed, the other not as yet in evidence. 16. Two well-developed hypobasidia at tip of hypha. 17. Normal development of two epibasidia from hypobasidium. 18. Formation of first longitudinal septum diagonally. 19. Early appearance of epibasidia

before septa are formed. 20. Four-nucleate epibasidia. 21 to 23; 25 to 27, 33 to 35. Abnormal septations of hypobasidia. In figure 33 no septa have been formed, while in figure 35 nucleus of lower cell is in prophase condition. 24. Immature gloeocystidium with much vacuolate protoplasmic content. 28. Uninucleate spores. 29 to 32. Germinating spores, showing variations of germ tube.

1. Whelden, R. M. Cytological Studies in the Tremellaceae III. Sebacina. Mycologia (In press).

A New Carex Hybrid.—Sometime during the winter of 1932–33, I received an exchange packet of plants from Professor Alfred S. Goodale of Amherst College. One of these was no. 68660, labelled *Carex pallescens,* from Woodstock, Grafton County, New Hamphsire, collected June 21, 1932. It did not look "right" to me, so I laid it aside for further study at the Gray Herabrium, after concluding it was a hybrid. Upon showing it to Professor Fernald, he suggested that it might be *C. hirtifolia* × *pallescens.* Critical study would seem to show that this "educated guess" was entirely correct. The leaves are as long, as broad, and as softly pubescent as in luxuriant *C. hirtifolia.* In technical characters the perigynia are exactly as in *C. pallescens,* but the scales tend to be more truncate, as in *C. hirtifolia.* The pistillate spike as a whole is, however, a combination of the two species, as it is larger and more long-cylindric than in *C. pallescens.* The staminate spike is well developed and 2 cm. long, much as in *C. hirtifolia.* At the same time Professor Goodale sent another sheet to the New England Botanical Club. Both presumed parent species occur in the Woodstock region. The hybrid origin of the collection seems to be the best interpretation of the facts at the moment. Certainly no sheet of *C. pallescens* in the enormous series in the Gray and the New England Club herbaria shows any such extreme of variation. —Ludlow Griscom, Harvard University.

Volume 37, no. 434, including pages 33–76 and plate 326, was issued 8 January, 1935.